ab111750

Protease Activity Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Protease activity in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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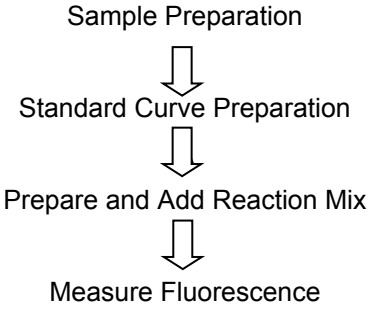
1. Overview

Proteases are naturally present in all organisms. These enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades. Proteases can either break specific peptide bonds (limited proteolysis), depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids (unlimited proteolysis). The activity can be a destructive change (abolishing a protein's function); an activation of a function (pre-form to mature form) or it can be a signal in a signaling pathway.

Abcam's Protease Activity Assay Kit is designed for the quantitative determination of proteases present in the protein sample. The assay uses fluorescein isothiocyanate (FITC)-labeled casein as a general protease substrate. The fluorescein label on the FITC-Casein is highly quenched. Upon digestion by proteases present in the sample the FITC-Casein substrate is cleaved into smaller peptides which abolish the quenching of the fluorescence label. The fluorescence of the FITC-labeled peptide fragments is measured at Ex/Em = 485/530 nm.

The kit is supplied with our Mass Spectrometry Grade (MSG), chemically stabilized Trypsin for use as a general protease control. However, other protease standard controls can also be used. This kit is easy to use and can detect <500 pg/well Trypsin present in the sample.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer I/Assay Buffer	25 mL
Protease Substrate/Protease Substrate (Lyophilized)	1 vial
Fluoresence Standard VI/FITC Standard (25 µM)	200 µL
Protease Positive Control/Positive Control (Lyophilized)	1 vial

* Store kit at -20°C, protect from light.

- Warm the Assay Buffer I/assay buffer to room temperature before use.
- Briefly centrifuge vials before opening.
- Read the entire protocol before performing the assay.

PROTEASE SUBSTRATE: Reconstitute with 220 µl dH_2O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.

Protease Positive Control/POSITIVE CONTROL: Reconstitute with 100 µl Assay Buffer I/Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Use within two months. Avoid freeze/thaw cycles.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. **For serum samples:** Serum samples can be directly diluted in the Assay Buffer I/Assay Buffer.

b. **For tissue or cell samples:** Tissues or cells can be extracted with 4 volumes of the Assay Buffer I/Assay Buffer, centrifuge to remove insoluble material and get a clear extract.

Prepare **test samples** up to 50 µl/well with Assay Buffer I/Assay Buffer in a 96-well plate.

For **positive** control use 5µl of the reconstituted Protease Positive Control/Positive Control solution into wells and adjust volume to 50 µl with Assay Buffer I/Assay Buffer.

Include a reagent **background control** which only contains 50 µl of Assay Buffer I/Assay Buffer.

Note: This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.

We suggest testing several doses of your sample to make sure readings are within the standard curve.

2. Standard Curve Preparation:

Add 0, 2, 4, 6, 8, 10 µl Fluoresence Standard VI/FITC Standard into a series of standards wells. Adjust the final volume to 100 µl/well

with Assay Buffer I/Assay Buffer to generate 0, 0.05, 0.1, 0.15 0.2, and 0.25 nmol/well of the Fluoresence Standard VI/FITC Standard.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

Assay Buffer I/Assay Buffer	48 µl
Protease Substrate Solution	2 µl

Add 50 µl of the Reaction Mix to each well containing the Positive Controls, Reagent Background Control and Samples. Mix well.
(DO NOT ADD TO STANDARDS)

4. Measurement: Read Ex/Em = 485/530 nm R_1 at T_1. Read R_2 again at T_2 after incubating the reaction at room 25°C for 30 min (or longer time if the sample activity is low); protect from light. The fluorescence of the unquenched FITC generated by proteolytic digestion of the substrate is:

$$\mathbf{\Delta RFU = R_2 - R_1}$$

Notes:

a) It is essential to read R_1 and R_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics, and then choose R_1 and R_2 in the reaction linear range.

b) Since the assay is a fluorescence quenching assay, the background reading is high, but sample reading are consistent.

5. Data Analysis

Subtract the zero standard from all standard readings.

Plot the Fluoresence Standard VI/FITC Standard Curve and apply the ΔRFU to the Standard Curve to get B nmol of FITC generated between T_1 and T_2 in the reaction wells.

Protease activity can then be calculated:

$$\text{Protease Activity} = \frac{\text{(B x Dilution Factor)}}{(T_2 - T_1) \times V} = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

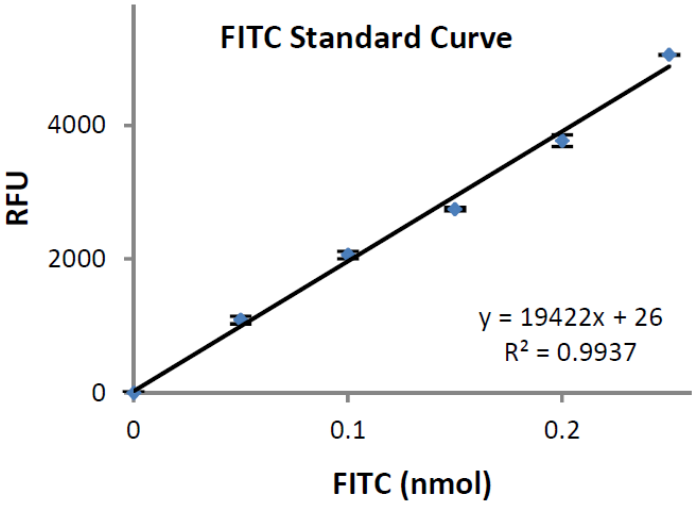
B is the FITC amount (nmol) from the Standard Curve

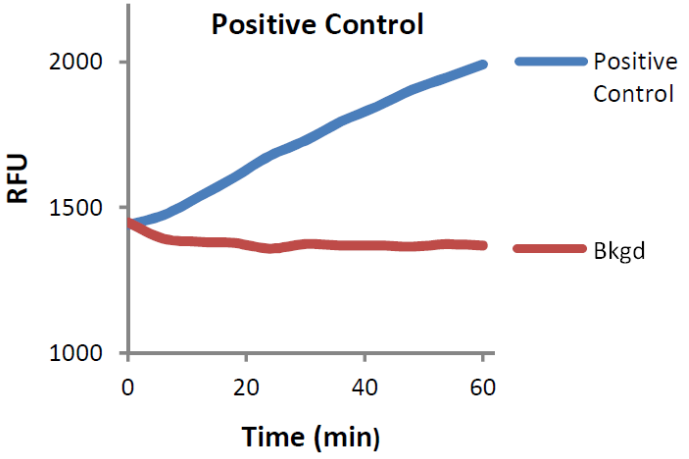
T_1 is the time (min) of the first reading (R_1)

T_2 is the time (min) of the second reading (R_2)

V is the pretreated sample volume (ml) added into the reaction well

Unit Definition: One unit is defined as the amount of protease that cleaves the substrate, to yield an amount of fluorescence equivalent to 1.0 µmol of unquenched FITC per minute at 25°C.


FITC Standard Curve
RFU
4000
2000
0
0
0.1
0.2
FITC (nmol)
y = 19422x + 26
R² = 0.9937


Positive Control
RFU
2000
1500
1000
0
20
40
60
Time (min)
Positive Control
Bkgd

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms) Luminescence: White plates Colorimetry: Clear plates If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/temperature	Refer to datasheet for recommended incubation time and/or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

7. Notes

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp